Table 2. Myeloid panel antigens distinct from Mac-1, LFA-1 family

Workshop no.	$M_r \times 10^{-3}$	Gran/U937
213, 248, 285, 328, 336, 337	160	−/+
236, 237, 280, 284, 295, 296, 305, 316, 317, 320, 321	240, 220, 190, 170, 160, 105	+/−
205	240	−/+
201, 214	185	+/?
206, 223	145	−/+
222	115	−/+
269	108	+/−
306	60	+/−

Table 3. Non-lineage panel antigens (U937 cells)

Workshop no.	$M_r \times 10^{-3}$
358, 360, 366, 385, 387, 390, 391, 392, 411, 414	240
405, 413	145
384, 386, 401	120

N2.3 Epitope masking of CD18 antigen

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LFA-1, CR3 (Mac-1 antigen), and CR4 (p150, 95) constitute a family of heterodimers each of which has a different alpha subunit (CD11a, b, and c, respectively) non-covalently associated with a common beta subunit (CD18).

H52, an anti-beta subunit antibody, was found unexpectedly to bind only the subunits of LFA-1. A cross-over experiment was performed in which a mixture of all three molecules was passed over an H52 column followed by an another anti-beta antibody, MHM23, and vice versa. H52 only recognized LFA-1, but the unbound CR3 and CR4 was recognized by MHM23. In contrast, MHM23 bound all three molecules and no LFA-1 passed through to be bound by the H52 column.

When dissociated CR4 was passed down an H52 column, the alpha subunit passed through and the beta subunit bound, indicating that H52 only recognizes the beta subunit of dissociated CR4. It was concluded that the epitope of H52 on the beta subunit is masked by the alpha subunits of CR3 and CR4 but not by the alpha subunit of LFA-1.

The leucocyte function associated antigen 1 (LFA-1/CD11a), complement receptor type 3 (CR3 or Mac-1 antigen/CD11b), and complement receptor type 4 (CR4 or p150, 95/CD11c) each have a specific alpha subunit (170, 160, and 150kDa, respectively) with a beta subunit (95kDa/CD18) common to all three members of the family [1]. All appear to be involved in cell adhesion reactions.

LFA-1 is involved in maintaining effector–target cell contact in cytotoxic lymphocyte-mediated killing [2] and other leucocyte–leucocyte interactions, for example T-cell-dependent antigen presentation to B-cells [3].

CR3 is a receptor for the inactivated form of bound C3, the iC3b fragment [4], identified by inhibition of iC3b-mediated rosetting [5] and formation of a ternary ligand–receptor–antibody complex [6]. In addition, it has been reported that CR3 has a lectin-like affinity for some polysaccharides [7].

CR4 was initially identified as a third molecule precipitated by anti-beta antibodies [1] with unknown function. Recent reports have indicated that p150, 95, as it was termed, specifically recognizes the iC3b fragment in a cation-dependent way that is similar to CR3 [8, 9]. Whether CR4 can mediate rosetting with C3-fragment-coated cells is not yet clear [9, 10].

Several anti-beta antibodies (CD18) have been described [1, 6, 11, 12, 13] which characteristically immunoprecipitate four peptides (the three alpha subunits and the beta subunit) from samples containing all

three molecules. Of these, H52 [12] is particularly useful, as its epitope is not irreversibly altered by the acid or alkaline conditions used for antigen elution. This allows the separation of alpha and beta subunits following immunoaffinity chromatography. It was during such a preparation that the inability of H52 antibody to recognize CR3 and CR4 was observed.

Immunoaffinity columns of MHM23 and H52 anti-beta-subunit monoclonal antibodies were prepared by coupling the purified monoclonal immunoglobulin to CNBr-activated Sepharose 4B. An antigen source was prepared by solubilizing normal human spleen membranes in a non-ionic detergent. The extracts were applied to the columns connected in tandem, in one experiment with the MHM23 column first, in the second with the H52 column first. The two columns were then separated and the non-specifically bound material was removed by washing with high ionic strength buffer. The specifically bound material was eluted with a low pH buffer. Figure 1 shows SDS–polyacrylamide gels of the eluted material. When placed first the MHM23 column binds four peptides, of molecular weight 170, 160, 150, and 95 kDa (Fig. 1, track 1). The three largest peptides were identified as the alpha-subunits of LFA-1, CR3, and CR4 respectively by pre-absorption with the relevant antibody column (data not shown). The smallest peptide is the beta-subunit. The H52 column when placed first only binds two peptides, the subunits of LFA-1 (Fig. 1, track 2).

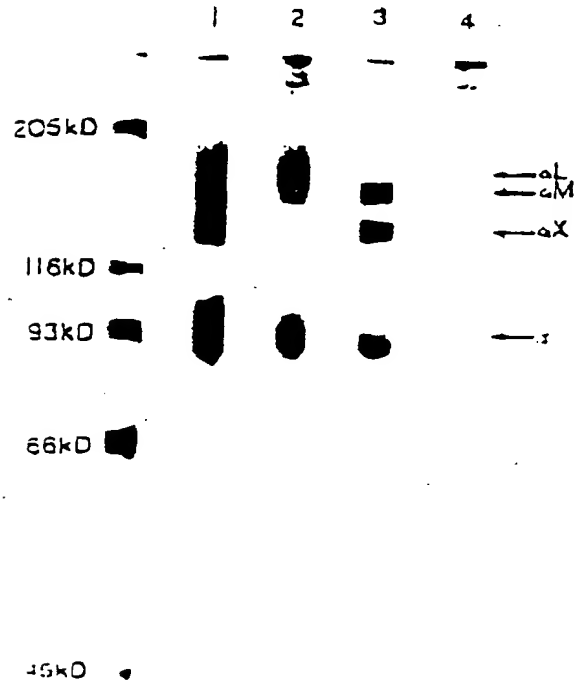

Fig. 1. Immunoaffinity chromatography of spleen membrane extracts (equivalent to 3 g tissue) on MHM23 and H52 columns (5 mg immunoglobulin bound to 1 ml CNBr-activated Sepharose). Flow rate 2 ml/h. Antigens were eluted with pH 2.2 buffer and the eluate was prepared for SDS–polyacrylamide gel electrophoresis by TCA precipitation, acetone washing, and solubilization in urea, SDS, and DTT. The gels were stained with Coomassie blue. Molecular weight standards are indicated on the left.

Track 3 of Fig. 1 shows the material bound to the MHM23 column when placed after the H52 column. Only three peptides are visible, corresponding to a mixture of CR3 and CR4. The leading H52 column has absorbed all the LFA-1 present. Track 4 shows the eluate from the H52 column placed after the MHM23 column. No material is bound, indicating that the MHM23 column has removed all the material recognized by H52.

These results indicate that H52 only recognizes the beta subunit of LFA-1. H52 has been characterized as an anti-beta-subunit antibody [13], but it must be assumed that the epitope of H52 is not expressed on the beta subunit of CR3 and CR4. There are two possible explanations for this: either the beta subunit of LFA-1 is different from that of CR3 and CR4 and thus H52 only recognizes the LFA-1 type, or that in some way the H52 epitope is masked in the CR3 and CR4 molecules. The latter possibility can be tested by investigating whether the H52 epitope is present on dissociated beta subunits derived from CR3 or CR4.

Solubilized spleen membranes were passed down an anti-CR4 (KB90) column as described for the anti-beta subunit antibodies. The eluted material was subjected to high pH to dissociate the subunits and neutralized before passage down an H52 column. Figure 2 shows the gels of the material eluted from the KB90 column (track 1), the material which passed through the H52 column (track 2), and the material eluted from the H52 column (track 3). The dissociated alpha subunit of CR4 passes through the H52 column (Fig. 2, track 2). The beta subunit is bound to the column (Fig. 2, track 3) indicating that the H52 epitope is expressed on the dissociated beta subunit.

It was concluded that the failure of H52 to recognize the beta subunits of CR3 and CR4 was due to the masking of the H52 epitope in the undissociated state. The simplest explanation is masking by the alpha subunit of CR3 and CR4, but not by the alpha subunit of LFA-1.

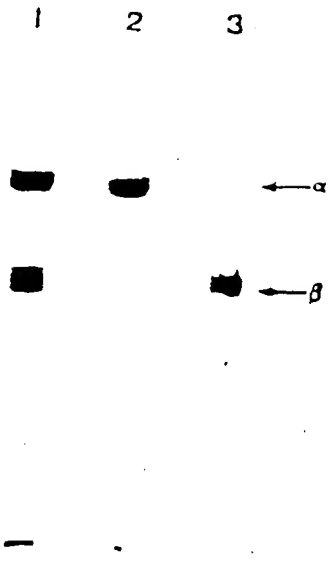

Fig. 2. Immunoaffinity chromatography of CR4. CR4 was prepared by affinity chromatography on K890 Sepharose as described for Fig. 1. The eluate was adjusted to pH 11.5 for 20 min with diethylamine. This was then gel filtered into neutral buffer and applied to an H52 column.

These results also explain the previously noted inability of H52 to immunoprecipitate CR3 [12].

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N2.3a Structural homology between the β-subunit of the human LFA-1/CR3/p150, 95 cell surface adhesion glycoproteins and a fibronectin binding protein, integrin, from chicken fibroblasts

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The complement receptor type 3 (CR3, or the Mac-1 antigen, or CD11b), the LFA-1 antigen (CD11a), and the p150, 95 antigen (CR4, CD11c) are members of a family of heterodimeric cell surface glycoproteins, each containing a unique α-subunit non-covalently associated with a common β-subunit (CD18). The primary structure of the β-subunit was studied by a combination of protein and cDNA sequencing. The sequence of one segment of the subunit showed that it contains three repeating units, each of which is about 40 amino acid residues in length and contains eight cysteine residues. This structure is highly homologous to that of the chicken integrin protein, which is presumed to form a transmembranous bridge between extracellular fibronectin and the intracellular actin molecules. It is speculated that the β-subunit may be a communication molecule across the

Leucocyte Typing III

White Cell Differentiation Antigens

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